Chemical Residues in Meat:

A large number of drugs used to control or prevent infections or to promote growth are considered essential in modern animal production. Additional chemicals may be added to food to ensure maximum utilization and to delay deterioration. However, there is growing consumer resistance to the presence of unwanted residues in food. Detection of these unwanted residues presents a new challenge to meat hygienists. Traditionally , meat inspection has involved a visual ante-mortem inspection of the animal and P.M examination of the carcass and offal. Many of the drugs used in modern production however, are rapidly absorbed or are given orally and therefore do not produce lesions that can be observed post-mortem. Therefore, to reassure consumers, traditional meat inspection procedures need to be complemented by an increasingly wide range of laboratory procedures.

A *residue* is defined as a substance having a pharmacological action and of a conversion products thereof and other substances transmitted to meat and which are likely to be dangerous to human health. Almost all chemicals administered to animals result in some trace residue remaining in the carcass. Increasingly, laboratory technology is able to detect these minute traces. It is therefore important to differentiate between safe and unsafe residual concentrations rather than to insist on zero residues.

Clearance rates for drug can vary. Conditions that prolong the process can lead to tissue residues at slaughter. Even when drugs are used according to recognized dosages and routes of administration and when pre-slaughter withholding times are observed; other parameters , e.g. disease conditions, age of animal and husbandry practices, can result in violative tissue

residues at slaughter. Drugs are also sometimes administered to food producing animals at a dose rate in excess of that recommended, by unauthorized routes or at more frequent intervals than specified. In all cases these therapies can alter the withholding time required to ensure that all tissues are clear of residues.

The pharmacokinetics (movement of drugs in the body) of specific preparations has a major effect on persistence in the animal tissue and is dependent on many factors. Formulation can give slow or rapid release. The chemical composition of some drugs prevent rapid metabolism. The route of administration , e.g by injection , orally or or other means , also affects the rate of excretion.

The therapeutic products that cause concern fall into a number of categories. The major ones are *antimicrobials* which are a diffuse group containing several classes of compounds used to treat or prevent bacterial infections. The *pesticides* are also a diffuse group including anthelmintics used for their activities against roundworms, tapeworms and fluke, ecto-parasiticides used to kill external parasites and antiprotozoals which are most commonly used for coccidiosis. *Hormones* are used for therapeutic purposes in various fertility treatments or for growth promotion when used by implant. One general category includes *tranquilizers and B – agonists.*

Animals are also exposed to many environmental contaminants including herbicides, heavy metals and fungicides. Some of these substances find their way into animal tissues via the feed.

In the preservation and processing of food, *additives* are employed to prevent the onset of spoilage , to promote binding properties and to enhance flavor and nutritive value. These additives include antioxidants, emulsifiers, humectants, firming agents, sequestrates, coloring agents, stabilizers, sweeteners, tenderizers, etc. at both production and processing stages,

residues or contaminants may enter the food chain either from intentional or accidental exposure to these chemicals.

For all residues it is essential to have an acceptable level in the diet. Calculation of the acceptable daily intake depends on the toxicology of the compound. These toxicological effects are determined by conducting studies involving genotoxicity, carcinogenicity, mutogenicity, teratogenicity, and effects on the immune and reproductive systems.

Structured surveys:

It is both economical and practically impossible to sample all carcasses for all residues. *Quality assurance* is therefore based on *sampling procedures* .

samples are collected from abattoirs in proportion to the annual throughput and on farms in proportion to the stock numbers. To avoid subjective selection of animals for sampling, a program that generates a list of random numbers is used.

The officer in the Meat plant is provided with a list of the samples that are required for his plant, and at a specified time , samples are taken from the next slaughtered animal of the species and type specified. The number of samples required from a species or type is chosen to ensure detection of a problem that affect a specific proportion of the population.

Maximum residue levels:

No chemical is safe under all conditions of use. It is therefore important that all are fully evaluated for safety either as the parent compound or as metabolites and that these determine acceptability.
Toxicological studies involve both acute toxic effects of the chemicals and more chronic effects including carcinogenesis and mutagenicity. Increasingly, studies of fertility and fetal

development and the effect on the immune system have been added to these assessments of safety.

As international markets become increasingly harmonized, standardization of acceptable residue levels is required. The maximum residue level (MRL) is a concept developed to estimate the maximum acceptable human intake over a lifetime.

It is generally accepted that the MRL of an analyte of any foodstuff is determined by three factors:
1. A minimum dose which produces detectable effects in experimental animals or which, in a therapeutic preparation used in human medicine, produces a recognizable effect.
2. a safety factor in the range of 10: 1000 and which is lower (1:100) if a preparation is already acceptable in human medicine or higher (1:1000) if there is any evidence to indicate a special risk from experience with chemically similar compounds.
3. a series of factors to balance the proportions of the particular tissues in the average diet.
The MRL is a figure set for acceptable or tolerable intakes believed, on the evidence available , to be safe for man but which may be modified upwards or downwards in the light of any new toxicological hazards to humans.

Acceptable daily intake:
It is an estimate of the amount of a food additive, expressed on a body weight basis , that can be ingested daily over a lifetime without appreciable health risk.

Antimicrobials :

The most numerous and most frequently used drugs in this group are the *antibiotics*. An antibiotic is a chemical substance, produced wholly or partly by a micro-organism (usually a fungus or bacterium) which has the capacity to inhibit the growth of or to kill bacteria.

These drugs can be used therapeutically in short courses of treatment to control disease in animals or, at lower concentration but over a longer time , to promote growth. The latter use occurs most frequently in young calves and poultry.

In the adult ruminant, alterations in the rumen flora may reduce efficiency of digestion , growth and weight gain. When used therapeutically antibiotics can reduce the symptoms of the disease and may result in a failure to identify unhealthy animals at ante-mortem inspection.

Antimicrobials are a difficult group to detect chemically because they are diverse and show great variation in their chemical weights. They are also used in a wide range of formulations and are administered by many routes. A common characteristic is their antimicrobial activity and this has been used to develop test systems. However, not all antibiotic residues retain activity after metabolism in animal tissue.

Commonly used antimicrobial agents include the penicillins, the aminoglycosides (dihydro-streptomycin, streptomycin and neomycin), chloramphenicol, tetracyclines, (chlortetracycline, oxytetracyclin, tetracycline), tylosin, sulphonamides and furazolidone. The cephalosporins, spiromycin and erythromycin are of increasing importance for specific diseases. Nystatin and griseoflavin are useful fungicides and a range of other agents , avoparcin, virginiamycin, polymyxin B , bacitracin, nitrofurazone and some sulphonamides , have been used as additives in feed. The latter result in growth promotion and are considered economically worthwhile when incorporated at non-therapeutic concentrations. Examples of actual and proposed maximum residue levels in meat is shown in the below table.

Table : Antibiotic maximum residue levels that have been proposed for meat.

Compounds	concentration(ng/g)
Apramycin	100
Bacitracin	0.7(IU/g)
Cephalosporins	60
Clopidol	200
Dihydrostreptomycin	1000
Erythromycin	300
Lincomycin	200
Neomycin	500
Novobiocin	500
Nystatin	7100
Oleandomycin	300
Polymyxin B	5(IU/g)
Spectinomycin	500
Spiramycin	25
Streptomycin	1000
Penicillin	60
Ampicillin	50
Amoxycillin	50
Benzylpenicillin	50
Cloxacillin	300
Chloramphenicol	10
Dapsone	25
Nitrofurans	5
Dimetriadazole	10

Sulphonamides	100
Trimethoprim	50
Oxacillin	300
Tetracycline	500
Chlortetracycline	50
Oxytetracycline	250
Virginiamycin	100
Tylosin	200
Tiamulin	400
Monensin	50
Lasolocid acid	700
Griseofulvin	200

Antibiotic residues are considered undesirable for several reasons. They produce unsightly lesions when administered by injection. The sight of the injection is discolored, and may be hemorrhagic if treatment was administered shortly before slaughter. In many of these cases the antibiotic is still present in an unmetabolized form. Long standing injection sites, particularly those incorporate an oily base, may be hard fibrous nodules within a muscle. The tetracycline, when given as long acting preparations, may leave a yellowish stained area. Since these lesions result in trimming by inspectors, it is prudent to administer injectable antibiotics always in non edible areas. During meat inspection all carcasses with injection sites should be retained and judgments made according to case history, the time of treatment and laboratory results. Frequently, there is no history of previous therapy, so the best evidence on which to base a judgment is the visual appearance of the lesion and the laboratory result.

Antibiotics may interfere with further food processing if this depends on fermentation reaction. They may cause allergic reactions in sensitized consumers. A small number of antimicrobials are suspected of having carcinogenic properties. There is also considerable concern regarding the creation of

resistant bacteria in farm animals which may then pass to the consumer. Studies have frequently demonstrated that subtherapeutic feeding of antimicrobials to livestock and poultry increases the prevalence of R+ enteric organisms some of which may be pathogenic for consumers.

The most frequently used tests for antimicrobial agents are based on detection of residual antimicrobial activity. The basic microbiological method is the four plate test (FPT) . it is an agar diffusion test. Meat samples are applied to four plates of agar medium, three of which are inoculated with Bacillus subtilis spores at pH 6 , 7,2 and 8 or Micrococcus luteus at pH 8. Trimethoprim is incorporated into the pH 7.2 medium to enhance the sensitivity of the test for sulphonamide residues. Diffusion of the active antibiotic is detected by the formation of zones of inhibition on one or more plates after overnight incubation. The reliability and sensitivity of the test is monitored by applying 6 mm-diameter filter paper discs containing standard quantities of known antibiotics in each turn.

Further evidence of the identity of the specific antibiotic can be obtained using high voltage electophoresis (HVE) bioautography. Two gels, agar and agarose are prepared, a piece of meat is placed on each and the antibiotic is allowed to diffuse into the medium. The high voltage is passed through the medium for a period of 2.5 hrs. the plates are then over-laid with media containing sensitive species of bacteria similar to those in the FPT and incubation is carried out overnight. The antibiotics inhibit the growth of bacteria over the area in which they are concentrated.

Hormones:

Hormones have been used for a variety of therapeutic and growth –modifying purposes in animals. They are particularly important group because of the reports from toxicological experiments claiming to show that they may be associated with

cancer. The most commonly cited example is diethylstilbestrol therapy given to pregnant mothers with threatened miscarriages. A significant proportion of girls born after this therapy subsequently developed cervical adenocarcinomas.
When the ban was first introduced , producers had access to other growth promoting hormone implants (trenbolone, zeranol, natural hormones) which gave better responses at slightly higher costs.
Tests for growth promoting hormones have been implemented, samples are taken from animals that are suspected of having been implanted when these are presented for slaughter. In the live animal which is tested on farm, blood or feces are the most convenient samples to collect. At slaughter, blood , rectal feces, liver, kidney and muscle can be obtained from all animals.
Screening tests for residues of hormonal growth promoters are based on immuoassays. These tests are rapid , sensitive, selective and cost-effective. Examples of typical limits of decision for these assyas are shown in below Table.

Table : Typical limits of decision for recognized growth-promoting hormones.

Compound	matrix	limit of determination (ng/g)
Diethylstilbestrol	urine	0.5
Hexoestrol	urine	0.5
Zeranol	serum	0.2
Trenbolone	bile	1.4
Estradiol	serum	0.03 (male)
Progesterone	serum	0.5 (male)
Testosterone	serum	0.3 (female)
Nor-testosterone	serum	0

The critical component of each assay is the antibody. These antibodies are prepared by linking the hormone to a larger protein molecule, thereby creating an immunogen which , when

injected into laboratory animals , elicits an immune response. High-affinity antibodies can be produced which, when diluted , result in very sensitive and selective tests.

Pesticides:

Pest control chemicals must be toxic to some living organisms to fulfill their role. Depending on the pest being controlled they may be termed insecticides, fungicides, etc. The insecticides that are directly applied to food animals and the anthelmintics are regarded as the most important subgroups. A number of MRLs for common pesticides are shown in below Table.

Table : proposed MRLs of pesticides

Pesticide	MRL (ppm)
Aldrin/dieldrin	0.2
Chlordane	0.05
Carbendiazim	0.1
DDT	1
Dichlorvos	0.05
Diflubenzuron	0.05
Endrin	0.05
Hexachlorobenzene	0.2
a-HCH	0.2
B-HCH	0.1
Y-HCH	1
Heptachlor	0.2
Diazinon	0.7
Chlorfenvinphos	0.2
Chlorpyrifosmethyl	0.05

HCH, hexachlorocyclohexane.

The chlorinated hydrocarbons are extremely durable, persistent and bio-accumulating compounds which find their way into the food chain usually through use in controlling environmental or

animal pests. They are, however, frequently more toxic in small amounts as their biological activity is greater. Following its introduction , DDT was one of the most successful synthetic insecticides and continued in general use for many years. However, the bio-accumulation that occurred in various food chains eventually resulted in the banning of the organochlorine pesticides by the 1970s.

The organophosphates (e.g. coumaphos, melathion, dichlorvos, diazinon) are exteremly toxic to mammals but are highly efficient insecticides. They are less persistent in the environment than organochlorines because they can be hydrolysed chemically and enzymically. A number of the members of this group can be taken up by plants and can enter the food chain unless proper pre-harvest precautions are taken. Several agrochemicals based on phenols are used as preservatives or herbicides.
Pesticides are detected by chemical techniques. In the laboratory , spectrographic methods of pesticide analysis using color producing reactions were the first to reach sensitivities at the ppm level but these methods have been replaced by chromato-graphic techniques.

Pesticides used to remove internal parasites such as liver fluke and nematodes are important in animal production systems. The salicylanide flukecides, oxyclosanide, closantel and rafoxanide are active against Fasciola hepatica. Thiabendazole was the first highly effective broad spectrum anthelmintics and are being used in the treatment of nematode infections. These anthelimintics are extensively metabolized in mammals after injection , they are generally short-lived and the metabolites predominate in the plasma, tissues and excreta.

In general these therapeutic drugs are used to control infections in farm animals therefore they are unlikely to be administered close to slaughter. A number are administered by injection which can be irritant, and many animals

require to be examined so that the active drug, if any residue remains, can be identified.

Heavy metals:

Excess intakes of heavy metals in food have caused many intoxications in man. These are most often caused by contaminated cereals or by accidental additions during processing but occasionally toxic concentrations occur in animal tissues and products. These can be associated with soils naturally high in the element or through environmental contamination from local industry. They may also occur from feeding grain treated with the toxic metal or from excess amounts remaining in the environment following previous use in paints, etc. These toxic chemicals are detected by atomic absorption spectrometry.

Lead:

Lead can accumulate in the tissues of animals grazing close to smelting plants or in animal ingesting paints or substances with high lead contents. Acute cases occur most commonly after ingestion of lead containing paint. During chronic exposure the metal accumulates in the bones but in more acute exposure the highest values are found in the liver and kidney.

Arsenic:

Arsenic is the second most important poisonous hazard for farm animals. They may be exposed to inorganic or organic arsenic compounds when they are given feed, forage or liquid contaminated with arsenical herbicides, rodenticides or insecticides. Chronic toxicity can occur when arsenical compounds are fed at low levels because the metal accumulates in the liver , kidney and bones.

Mercury:

It has been most frequently associated with feeding to animals seed grain treated with mercury –containing dressings to prevent fungal growth.

Cadmium:

In farm animals the greatest concentrations occur in kidney and liver. Kidney mal-function in man begins when the concentrations are above 200 ug/g wet weight.

Copper:

The metal tends to be accumulated in liver and kidney.

Other metals such as fluorine and selenium.

Natural toxins:

Mycotoxins are products of toxigenic moulds growing in food and foodstuffs. These agents have caused many problems in livestock and the potential for residues in meat, poultry or dairy products is a cause of public concern.
Aflatoxins are produced by Aspergillus flavus and Aspergillus parasiticus. There are four major types of toxin labeled AFB1, AFB2, AFG1 and AFG2. AFB1 is the most commonly produced and the most toxic. Liver, kidney and milk are considered to be the most vulnerable to residue accumulation.
Ochratoxins are produced by some Penicillium spp. And some Aspergillus strains. Ochratoxin A is the most common and the most toxic to birds , mammals and fish. The kidney is the site for
The presence of these toxins and they can be detected by a range of commercially –produced *immunoassay KITS* ,and if positive animals are identified, they should be retained on a toxin-free diet for 4 weeks prior to slaughter to ensure that the levels in kidney have decreased.